Supplementary Information

Hydrogen Sulfide-Activatable Prodrug-Backboned Block Copolymer Micelles for Delivery of Chemotherapeutics

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Materials and methods

Materials. Methyl 5-amino-2-methylbenzoate, di-tert-butyl decarbonate, trimethylamine (Et_3N), *N*-bromosuccinimide (NBS), azobisisobutyronitrile (AIBN), sodium azide, succinic acid mono(2-(2-methacryloyloxy) ethoxy) ester, *N*-hydroxy succinimide (NHS), 4-dimethylaminopyridine (DMAP), dicyclohexylcarbodiimide (DCC), trifluoroacetate (TFA), m-aminobenzoic acid, nile red, sodium hydrosulfide (NaHS), propargyl cysteine (SPRC), glutathione (GSH) and L-cysteine (Cys) were purchased from Energy Chemical Co., Ltd. (Shanghai, China). Paclitaxel (PTX) was purchased from Huilin Bio-tech Co., Ltd. (Xi'an, China). Methoxypoly (ethylene glycol) with a molecular weight of ~5000 ($mPEG_{5K}$-OH) was purchased from Sigma-Aldrich (Shanghai, China). 7-Azido-4-methylcoumarin (AzMC) and 3-(4,5-dimethylthiazole-2)-diphenyltetrazolium bromide (MTT) were purchased from Beyotime Institute of Biotechnology (Shanghai, China). Fetal bovine serum (FBS), penicillin-streptomycin solution, trypsin, and RPMI 1640 medium were purchased from GIBCO. NucRed Live 647 Ready Probes Reagent, Lyso Tracker Green and Hoechst 33342 were purchased from Invitrogen. All other solvents were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai China). 4-cyano-4-trithiophenethyl valeric acid (PETTC) was synthesized according to the literature[1].

Human colon cancer cell line CT26 cells and human breast cancer cell line MDA-MB-231 cells were purchased from American Type Culture Collection (Manassas, USA). Cells were incubated in RPMI1640 medium containing 10% heat-inactivated FBS and 1% penicillin/ streptomycin solution in a humidified atmosphere of 5% CO_2 at 37 °C.

Female BALB/c mice (6–8 weeks) and nude mice were purchased from the Animal Center of Zhejiang University and maintained under standard conditions. Animal experiments were approved by the Animal Care and Use Committee of Zhejiang University and were carried out in accordance with the institutional guidelines.

Analytical methods. The ^1H NMR spectra of each chemical intermediate and the final

products were recorded on a Bruker Avance DRX-400 spectrometer at 400MHz with $CDCl_3$ as solvent and tetramethylsilane as the internal standard. Gel penetration chromatography (GPC) was performed on a Wyatt GPC/SEC-MALS (Wyatt Technology Corporation, Santa Barbara, USA) system equipped with an Optilab® T-rEXTM refractive index detector at 50 ºC using DMF containing 50 mM LiBr as eluent at a flow rate of 0.80 mL/min. Data were recorded and processed with ASTRA v6.0 software. High performance liquid chromatography (HPLC) was recorded on an Agilent 1260 Infinity II (Agilent Technologies) system equipped with an UV and FLV detectors using acetonitrile/water as eluent. Data were recorded and processed with software. Matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) was performed on a Bruker ultrafleXtreme™ MALDI-TOF mass spectrometer (Bruker Daltonics GmbH, Bremen, Germany) using 2,5-dihydroxybenzoic acid (DHB) as the matrix. Spectra were analyzed with FlexAnalysis v3.4 software. The sizes and zeta potentials of micelles were measured by on a Malvern Nano ZS Zetasizer (Malvern Instrument Ltd., UK) with He-Ne laser light (632.8 nm) at 173° scattering angle at 25 °C. The volume-averaged diameter was obtained from the instrument's Dispersion Technology Software version 6.1. The morphologies of micelles were observed using a JEM-1200EX TEM operated at a voltage of 80 kV. Micelles solution (1 mg/mL) was applied onto a 150-mesh copper grid and stained with 1% (w/v) aqueous uranyl acetate before observation.

Synthesis of PEG-PAMPTX

Synthesis of compound 2. Methyl 5-amino-2-methylbenzoate (compound 1, 10.00 g, 60.54 mmol) and di-tert-butyl decarbonate (15.00 g, 68.76 mmol) were dissolved in 100 mL THF, followed by the addition of Et_3N (10 mL, 72.13 mmol). The reaction mixture was stirred at 45 ºC until the complete reaction of compound 1 monitored by TLC. The solvent was removed by rotary evaporation, and the residue was dissolved in 200 mL ethyl acetate. The organic solution was successively washed with 1 N HCl, saturated $NaHCO_3$ aqueous solution, and brine, and then dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure, and the pure compound 2 was eventually obtained after column chromatography (n-hexane/ethyl acetate = 10/1, v/v) as a white solid (10.75 g, yield: 67.0%). ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.87 (d, J = 2.4 Hz, 1H), 7.45 (t, J = 14.9 Hz, 1H), 7.15 (d, J = 8.3 Hz, 1H), 6.58 (s, 1H), 3.86 (s, 3H), 2.52 (d, J = 13.4 Hz, 3H), 1.51 (s, 9H).

Synthesis of compound 3. Compound 2 (10.75 g, 40.54 mmol), NBS (7.93 g, 44.80 mmol) and AIBN (0.66 g, 4.02 mmol) were dissolved in 150 mL carbon tetrachloride. The reaction mixture was refluxed with *hv* irradiation under Ar atmosphere for 3 h. The solution was washed with water, saturated $NaHCO_3$ aqueous solution and brine successively, and then dried over anhydrous Na_2SO_4. The solvent was evaporated and the crude product was further purified by column chromatography (n-hexane/ethyl acetate = 10/1) to give compound 3 as a white solid (9.86 g, yield: 70.73%). ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.94 (d, J = 2.4 Hz, 1H), 7.55 (d, J = 6.8 Hz, 1H), 7.37 (d, J = 8.4 Hz, 1H), 6.68 (s, 1H), 4.92 (s, 2H), 3.92 (s, 3H), 1.51 (s, 9H).

Synthesis of compound 4. Compound 3 (9.86 g, 28.67 mmol) was dissolved in 50 mL DMF followed by the addition of sodium azide (2.23 g, 34.31 mmol). The reaction mixture was stirred at 45 ℃ for 1 h and then stirred overnight at room temperature. After complete reaction of compound 2, the reaction mixture was quenched with water. The solution was extracted with ethyl acetate (50 mL × 3), and the combined organic phase was washed with brine and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure to obta in pure compound 4 as a white solid (8.54 g, yield: 97.3%). ^{1}H NMR (400 MHz, $CDCl_3$), δ 8.01 (d, J = 2.4 Hz, 1H), 7.59 (dd, J =

8.2, 1.7 Hz, 1H), 7.38 (d, *J* = 8.4 Hz, 1H), 4.72 (s, 2H), 3.90 (s, 3H), 1.52 (s, 9H).

Synthesis of compound 5. Compound 4 (8.54 g, 27.91 mmol) was dissolved in a mixture of 50 mL methanol and 30 mL THF, and then 30 mL 10% NaOH aqueous solution was added. The reaction mixture was stirred at room temperature for 3 h. The organic solvent was removed by evaporation and the aqueous phase was washed three times with DCM. The aqueous solution pH was then adjusted to 1.0 and extracted with ethyl acetate (50 mL × 3). The combined organic solution was washed with brine, dried over anhydrous Na_2SO_4, and concentrated. The resulting residue was precipitated in n-hexane to give compound 5 as a white solid (7.01 g, yield: 86.0%). ^{1}H NMR (400 MHz, $CDCl_3$), δ 8.10 (d, *J* = 2.3 Hz, 1H), 7.68 (s, 1H), 7.43 (d, *J* = 8.4 Hz, 1H), 4.79 (s, 2H), 1.54 (s, 9H).

Synthesis of compound 6. Compound 5 (1.03 g, 3.46 mmol) was dissolved in a mixture of 10 mL DCM and 3 mL TFA and stirred overnight at room temperature. The solvent was removed, and the residue was dissolved in water. The solution pH was adjusted to 5.0 by adding saturated $NaHCO_3$ aqueous solution. The aqueous solution was extracted with ethyl acetate (50 mL × 3), and the combined organic phase was washed with brine, and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the pure compound 6 was obtained after column chromatography (n-hexane/ethyl acetate = 1/1) as a light-yellow solid (0.35 g, yield: 52.69%). ^{1}H NMR (400 MHz, D_2O), δ 7.93 (d, *J* = 2.3 Hz, 1H), 7.61 (dt, *J* = 8.3, 5.3 Hz, 2H), 4.75 (s, 2H).

Synthesis of compound 7. Mono-2-(methacryloyloxy)ethyl succinate (5.00 g, 21.74 mmol), NHS (5.00 g, 43.44 mmol), and DMAP (1.00 g, 8.15 mmol) were dissolved in 100 mL anhydrous CH_2Cl_2. DCC (6.70 g, 32.54 mmol) dissolved in 50 mL anhydrous DCM was added dropwise at 0 ℃. The reaction mixture was stirred overnight at room temperature. The solution was filtered to remove the white precipitate dicyclohexylurea. The filtrate was concentrated and another 200 mL ethyl acetate was added to dissolve the residues. The organic solution was successively washed with aqueous solutions of 1N HCl, $NaHCO_3$ and brine, and then dried over anhydrous Na_2SO_4. After concentration, the crude product was recrystallized in ethyl acetate/n-hexane to give mono-2-(methacryloyloxy)ethyl succinate succinimide ester (HEMA-

CONHS) as a white crystal (5.44 g, yield: 76.40%).

HEMA-CONHS (0.72 g, 2.20 mmol), compound 6 (0.35 g, 1.82 mmol) and DMAP (0.05 g, 4.08 mmol) were dissolved in 50 mL anhydrous DCM with 2 mL Et_3N, and the mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure, and the residue was dissolved in 100 mL ethyl acetate. The organic solution was washed with 1N HCl and brine, and then dried over anhydrous Na_2SO_4. After evaporation of the solvent, the pure compound 6 was eventually obtained by column chromatography (n-hexane/ethyl acetate = 1/1 by volume) as a light yellow solid (0.44 g, yield: 59.8%). ^{1}H NMR (400 MHz, $CDCl_3$), δ 8.32 (s, 1H), 8.10 (s, 1H), 7.95 (d, J = 8.0 Hz, 1H), 7.41 (d, J = 8.4 Hz, 1H), 6.12 (s, 1H), 5.58 (m, 1H), 4.75 (s, 2H), 4.37 (m, 4H), 2.78 (m, 4H), 1.92 (s, 3H).

Similarly, compound 9 was obtained as a white solid (yield: 59.7%). ^{1}H NMR (400 MHz, $CDCl_3$), δ 8.36 (s, 1H), 8.01 (m, 2H), 7.78(m, 1H), 7.38 (t, J = 7.9 Hz, 1H), 6.12 (s, 1H), 5.57 (m, 1H), 4.37 (s, 4H), 2.77 (dt, J = 30.9, 6.2 Hz, 4H), 1.92 (d, J = 6.7 Hz, 3H).

Synthesis of AMPTX. Compound 6 (0.44 g, 2.48 mmol), PTX (0.93 g, 1.08 mmol) and DMAP (0.05 g, 4.08mmol) were dissolved in 50 mL anhydrous DCM. DCC (0.45 g, 2.19 mmol) dissolved in 50 mL anhydrous DCM was added dropwise at 0 ℃. The reaction mixture was then warmed up to room temperature and stirred overnight. After filtration, the organic solution was successively washed with 1N HCl, saturated $NaHCO_3$ aqueous solution and brine, and then dried over anhydrous Na_2SO_4. The solvent was removed by evaporation, and the pure AMPTX was obtained by column chromatography (n-hexane/ethyl acetate = 1/1) as a white solid (0.84 g, yield: 61.54%). Similarly, HPTX was obtained as a white solid (yield: 55.2%).

Synthesis of PEG-PAMPTX. $mPEG_{5K}$-OH (10.00 g, 2.00 mmol), PETTC (2.00 g, 5.90mmol) and DMAP (0.40 g, 32.64 mmol) were dissolved in 100 mL anhydrous CH_2Cl_2. DCC (2.00 g, 6.66mmol) in 10 mL anhydrous CH_2Cl_2 was added dropwise at 0°C under Ar atmosphere. The reaction mixture was stirred overnight at room temperature. After filtration, the solvent was evaporated under reduced pressure and the residue was precipitated in cold ether for three times and dried under vacuum to give

$mPEG_{5K}$-PETTC as a light yellow solid (10.10 g, yield: 89.0%).

H_2S-responsive amphiphilic polymeric prodrug was synthesized via reversible addition-fragmentation chain transfer (RAFT) polymerization. Typically, $mPEG_{5K}$-PETTC (0.20 g, 0.036 mmol), AMPTX (0.20 g, 0.16 mmol), AIBN (0.65 mg, 0.004 mmol) and 1.5 mL DMF were charged into an ampule tube. The mixture was degassed by three cycles of freeze-pump-thaw, and sealed under Ar atmosphere. The reaction tube was placed in an oil bath at 75℃ for 12 h, and then quenched by dipping the tube in liquid nitrogen. The mixture was precipitated in cold ether three times and dried under vacuum to give PEG-PAMPTX as a light yellow solid (0.36 g, yield: 90.0%). Similarly, PEG-PHPTX was obtained as a light yellow solid (yield: 83.8%).

Synthesis of Cy5.5-labeled PEG-PAMPTX (PEG-$^{Cy5.5}$PAMPTX)

The amino-containing monomer was copolymerized with AMPTX according to the same procedure as described above except that 5% (molar ratio) of AMPTX was replaced with Boc-AMA monomer. The obtained PEG-P(AMPTX-*co*-Boc-AMA) polymer (100 mg) was then dissolved in 2 mL CH_2Cl_2 containing 1 mL TFA. After stirring at room temperature for 2 h, the solvent was removed by rotary evaporation. The residue was dissolved in 1 mL DMF, and Cy5.5-NHS (1 mg) and Et_3N (20μL) were added. The reaction mixture was stirred overnight at room temperature in the dark and then dialyzed successively against methanol and deionized water for 24 h. The PEG-$^{Cy5.5}$PAMPTX was then purified by a Sephadex G15 column and lyophilized to obtain a light green powder (76 mg, yield: 75.2 %).

Preparation of polymeric micelles

PEG-PAMPTX (10 mg) or PEG-PHPTX (10 mg) were dissolved in 1 mL DMF and then added dropwise into 10 mL deionized water with vigorous stirring. After 30 min, the solution was dialyzed against (MWCO 3500) deionized water (2 L × 5) for 24 h. The micelle solution was obtained after filtration through 0.22 μm membrane. The volume-averaged sizes and zeta potentials of the micelles were measured in triplicate using dynamic light scattering (DLS) on a NanoZS nanosizer (Malvern), and the morphology of the micelles was observed by TEM

Critical micelle concentration (CMC) determination

The CMCs of PEG-PAMPTX or PEG-PHPTX were determined via the

fluorescence method. Briefly, Nile red dissolved in CH_2Cl_2 (10^{-4} M, 30 μL) was placed in each vial. The organic solvent volatilized after exposure to the air in dark. Then micelles solutions (3 mL) of different concentrations were added into each vial to reach a Nile red final concentration of 10^{-6} M and shaken overnight at 37 ℃ in the dark. The fluorescence intensity of solutions in each vial was measured by a microplate spectrophotometer (SpectraMax M2E, Molecular Devices Inc., CA, USA) with 579 nm excitation and 620 nm emission. The CMC was defined as the intersection of the two fitting lines of the plots of fluorescence intensity versus the polymer concentration.

Assessment of micelle stability

The PEG-PAMPTX or PEG-PHPTX micelles (0.5 mM) were incubated in cell culture medium with 10% FBS at 37 °C for one week with shaking (200 rpm). The micelle stability in response to H_2S was measured in the presence of 10 mM NaHS. The size changes of micelles were monitored using DLS.

***In vitro* H_2S-triggered PTX release**

PEG-PAMPTX or PEG-PHPTX micelles (1 mL, PTX-eq. concentration 1 mg/mL) were sealed in a dialysis bag (3500 MWCO) and dialyzed against 50 mL of pH 7.4 PBS containing 0.5% Tween 80) with NaHS (10 mM, 5 mM or 1 mM), GSH (10 mM) or Cys (10 mM) at 37° C with shaking (150 rpm). At timed intervals, 100 μL of the dialysate was withdrawn for PTX determination using HPLC.

Cell cytotoxicity assay

The cytotoxicity of PEG-PAMPTX and PEG-PHPTX was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay against mouse CT26 colon cancer cells, and human MDA-MB-231 breast cancer cells. Their cytotoxicity in the presence of an intracellular H_2S inducer, S-(2-propynyl)-L-cysteine (SPRC), was also tested against the above cell lines.

Cells were seeded in 96-well plates (5000 cells/well) and followed by 24 h incubation. After 48 h of incubation with free PTX, PEG-PAMPTX, or PEG-PHPTX micelles at different concentrations with or without SPRC (1 mM), the medium in each well was replaced with 0.2 mL of fresh medium containing 0.5 mg/mL MTT and incubated for 4 h. Then the medium in each well was replaced with 100 μL DMSO to

dissolve the formazan crystals. The absorbance in each well was measured at 562 nm and 620 nm using a microplate spectrophotometer. Each drug concentration was tested in triplicate in three independent experiments. Cell viability was calculated according to the following formula.

$$\text{Cell viability} = \frac{OD_{\text{sample, 562nm}} - OD_{\text{sample, 620nm}}}{OD_{\text{control, 562nm}} - OD_{\text{control, 620nm}}} \times 100\%$$

Cellular uptake assays

The cellular uptake pathways of micelles were measured by flow cytometry using various endocytosis inhibitors . MDA-MB-231 cells were seeded in 12-well plates at a density of 1×10^5 in 1 mL medium and incubated overnight. The medium was replaced with 1 mL fresh medium. Each inhibitor, filipin (7.5 μM), wortmannin (5 μM), chlorpromazine (30 μM), or cytochalasin D (5 μM) was separately added to the medium and incubated with cells for 1 h, and then PEG-$^{Cy5.5}$PAMPTX micelles (Cy5.5-eq. 0.5 μg/mL) were added for incubation. The influence of temperature on the cellular uptake was performed at 4 °C. After 2 h-incubation, the medium was removed and the cells were rinsed with PBS, trypsinized, and then washed twice with PBS. The collected cells were resuspended in PBS and analyzed using flow cytometry. Every 10,000 cells were counted to determine Cy5.5-positive cell percentage at the FL4 channel. The experiment was repeated three times independently.

The cellular uptake was also observed by the confocal laser scanning microscopy (CLSM). MDA-MB-231 cells were seeded in a glass-bottom Petri dish at a density of 1×10^5 in 1 mL medium and incubated overnight. Then PEG-$^{Cy5.5}$PAMPTX micelles (Cy5.5-eq. 0.5 μg/mL) was added and incubated with cells. The lysosomes and nuclei were stained with LysoTracker Green and Hoechst 33342 for 0.5 h, respectively. At timed intervals, the cells were washed with PBS for three times and observed by CLSM. The excitation and emission wavelengths were 405 nm and 461 nm for Hoechst 33342, 488 nm and 511 nm for LysoTracker Green, and 640 nm and 694 nm for Cy5.5, respectively.

***In vivo* pharmacokinetics analysis**

Female ICR mice were randomized into 3 groups (n = 3). Taxol, PEG-PAMPTX,

or PEG-PHPTX (PTX-eq. 10 mg/kg) was injected intravenously (*i.v.*) into the mice via the tail vein. At timed intervals, blood samples (50 μL) were collected via the orbital venous plexus and then incubated overnight with 0.1 M acetic acid at 37 °C to liberate PTX. Then 1 mL of methanol was added to extract the free PTX and the mixture was centrifuged at 5000 rpm for 5 min. The supernatants were withdrawn and volatilized to dryness under N_2 flow; 100 μL of acetonitrile was then added to re-dissolve the residue, and the PTX content was measured by HPLC.

***In vivo* antitumor efficacy**

The orthotopic breast tumor model was established by subcutaneous inoculation of MDA-MB-231 cells (1×10^7 cells per mouse) into the mammary fat pad of BALB/c nude mice (6-8 weeks). When the tumor volumes reached ~100 mm^3, the mice were randomly divided into four groups (n = 6) and *i.v.* injected with PBS, Taxol, PEG-PAMPTX, or PEG-PHPTX at a PXT-eq. dose of 10 mg/kg following a q2d×5 regimen. The tumor volume and mice body weight were monitored individually every two days. The tumor volumes were calculated as V (mm^3) = L (mm) × W $(mm)^2$ × 0.5, where L is the longest length and W is the longest width of the tumor. The mice were sacrificed 22 days post treatment, and the tumors and major organs were dissected. The tumors in each group were weighed to calculate the tumor inhibition rate (TIR) according to the following formula: TIR = (mean tumor weight of the control group − mean tumor weight of the treatment group)/mean tumor weight of control group × 100%.

Histological assay

Tumor and major organ samples were washed with PBS, fixed with 4% neutral buffered paraformaldehyde, and embedded in para□n. Tissues were cross-sectioned into 10-μm-thick slices, stained with hematoxylin-eosin (H&E, Beyotime, China) and examined under optical microscopy.

Statistical analysis

Microsoft Office Excel 2016 and Graphpad Prism 8 software were used for processing data and statistics, and data are presented as mean ± SD. Comparisons were made using a two-tailed, unpaired Student's t-test. $P < 0.05$ was regarded as statistically significant.

Supplementary Figures

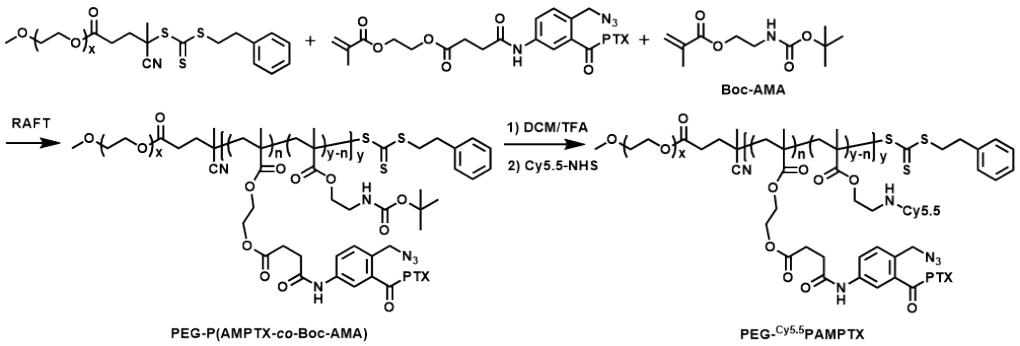

Fig. S1 The synthetic route of Cy5.5 labeled PEG-PAMPTX (PEG-$^{Cy5.5}$PAMPTX).

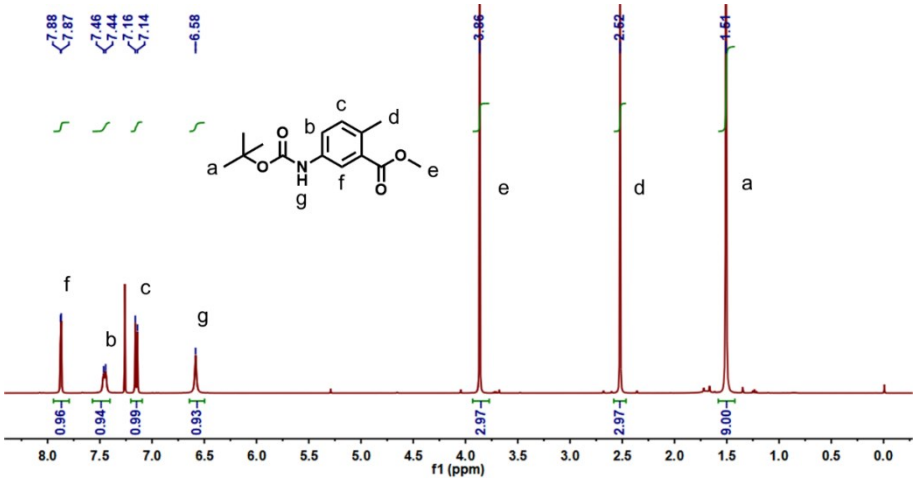

Fig. S2 The ^{1}H NMR spectrum of compound 2 in $CDCl_3$.

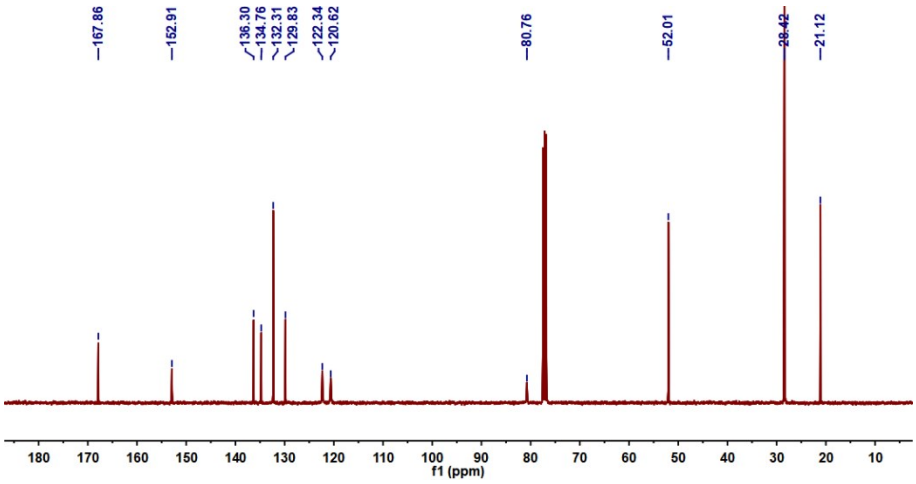

Fig. S3 The ^{13}C NMR spectrum of compound 2 in $CDCl_3$.

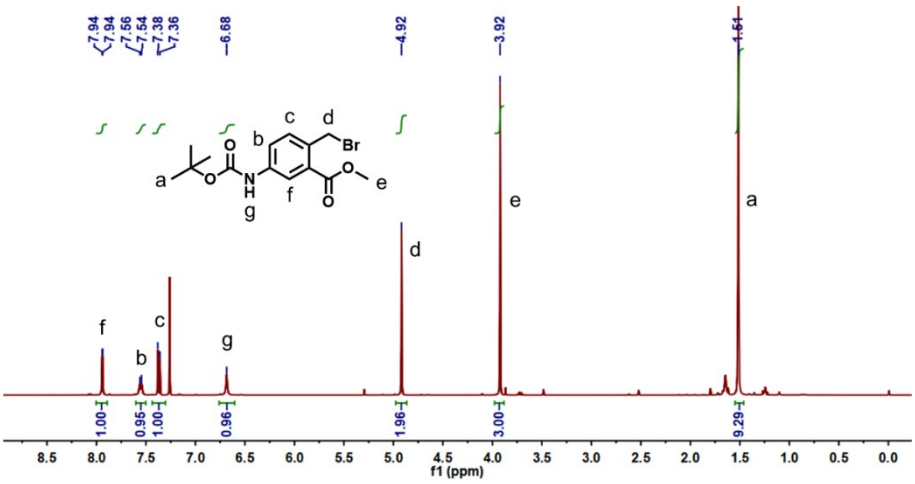

Fig. S4 The ^{1}H NMR spectrum of compound 3 in $CDCl_3$.

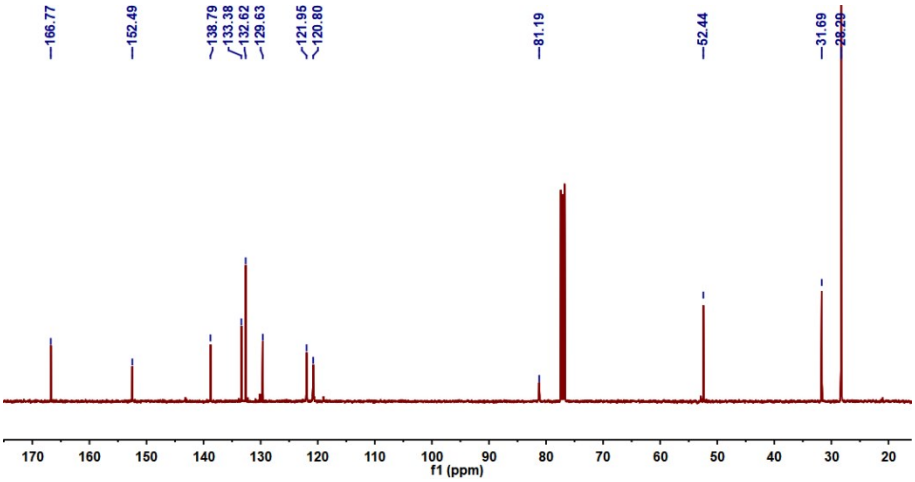

Fig. S5 The ^{13}C NMR spectrum of compound 3 in $CDCl_3$.

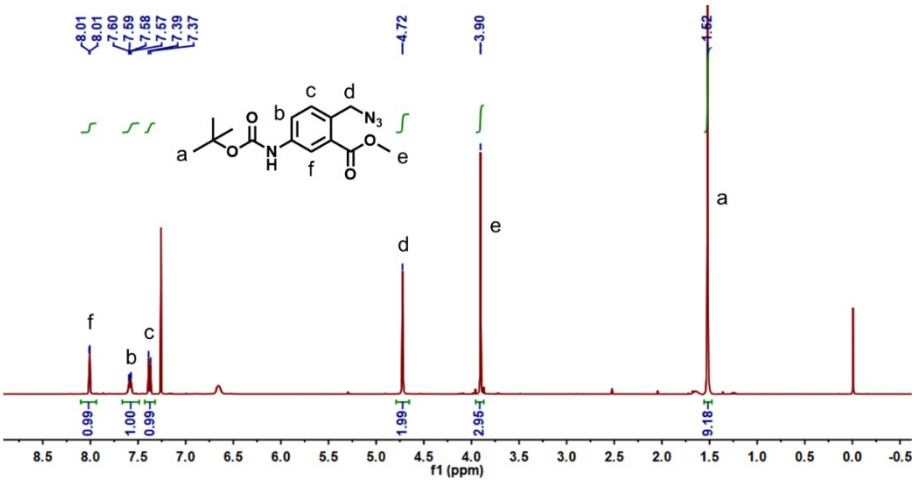

Fig. S6 The ^{1}H NMR spectrum of compound 4 in $CDCl_3$.

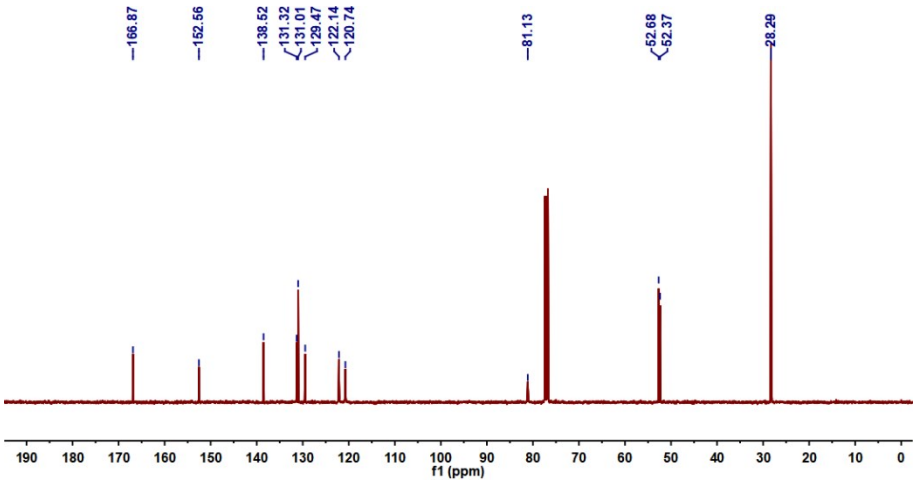

Fig. S7 The ^{13}C NMR spectrum of compound 4 in $CDCl_3$.

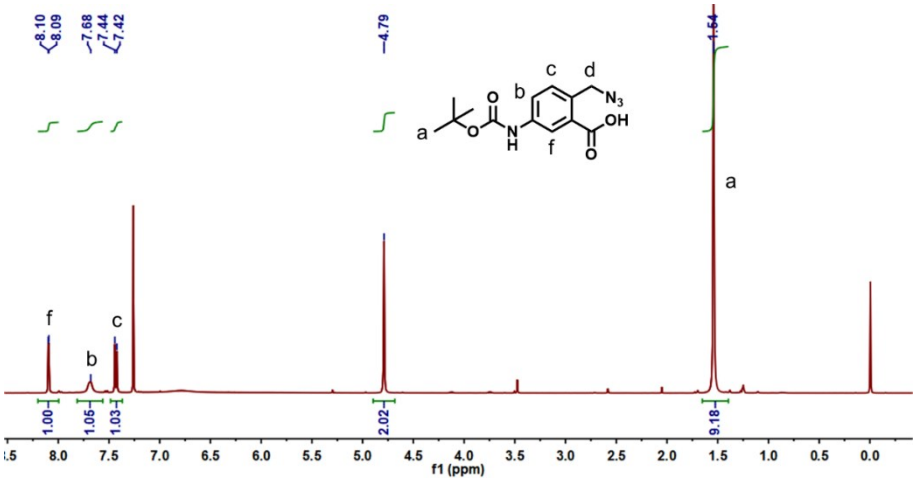

Fig. S8 The ^{1}H NMR spectrum of compound 5 in $CDCl_3$.

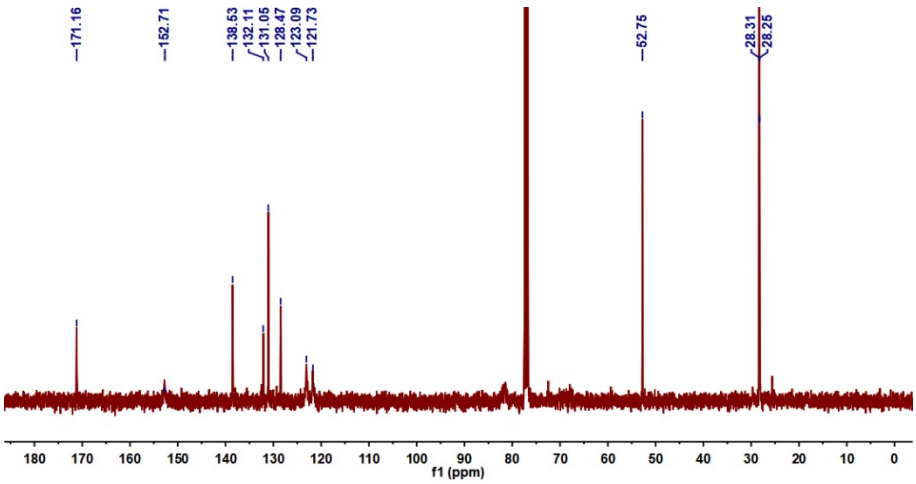

Fig. S9 The ^{13}C NMR spectrum of compound 5 in $CDCl_3$.

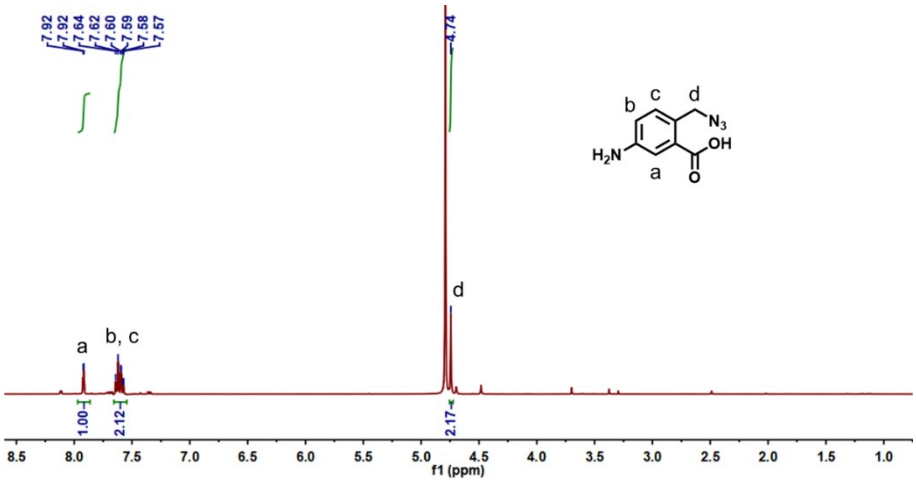

Fig. S10 The ^{1}H NMR spectrum of compound 6 in D_2O.

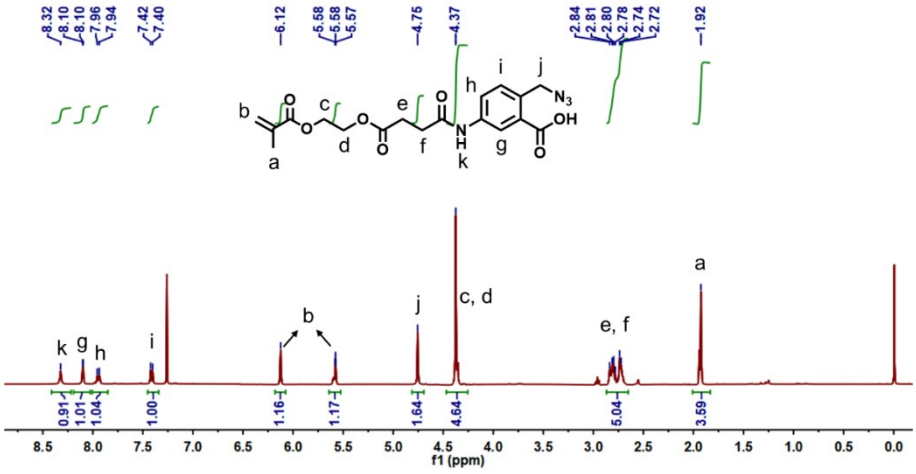

Fig. S11 The ^{1}H NMR spectrum of compound 7 in $CDCl_3$.

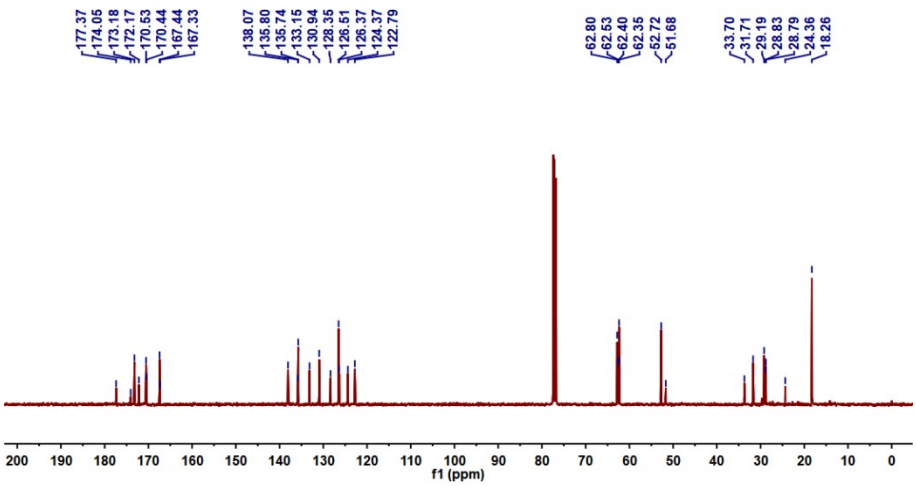

Fig. S12 The ^{13}C NMR spectrum of compound 7 in $CDCl_3$.

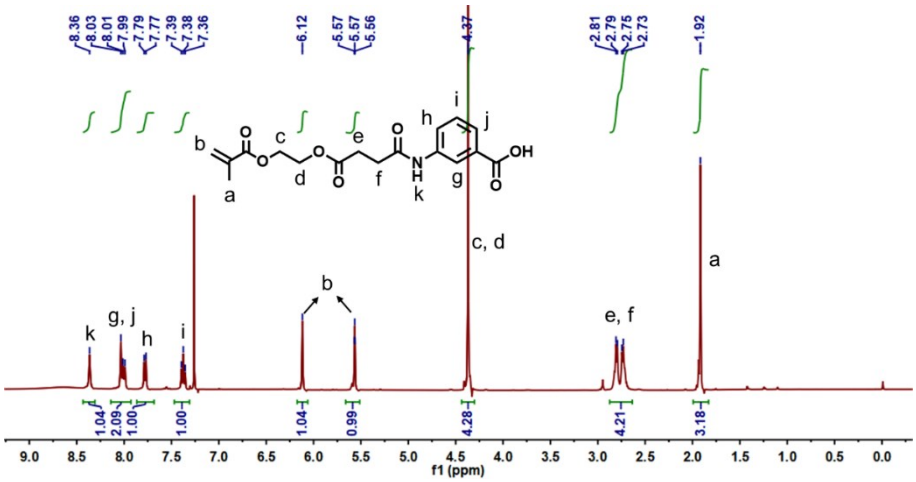

Fig. S13 The ^{1}H NMR spectrum of compound 8 in $CDCl_3$.

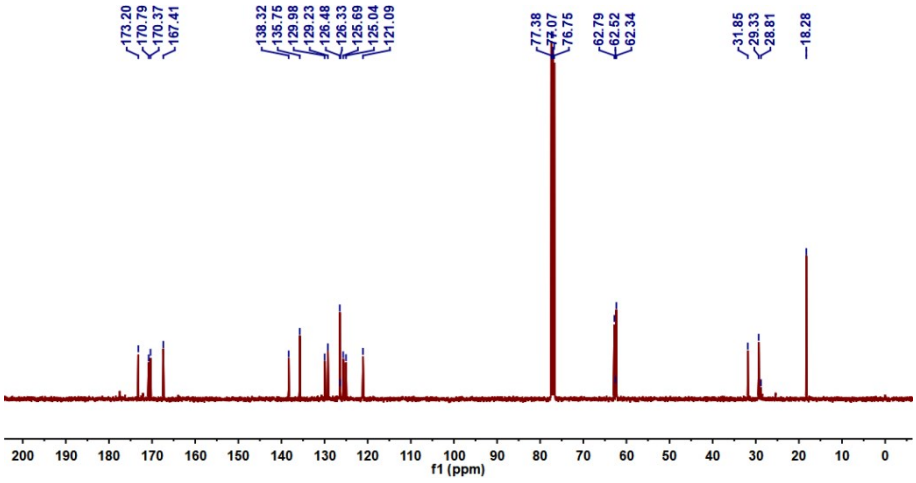

Fig. S14 The ^{13}C NMR spectrum of compound 8 in $CDCl_3$.

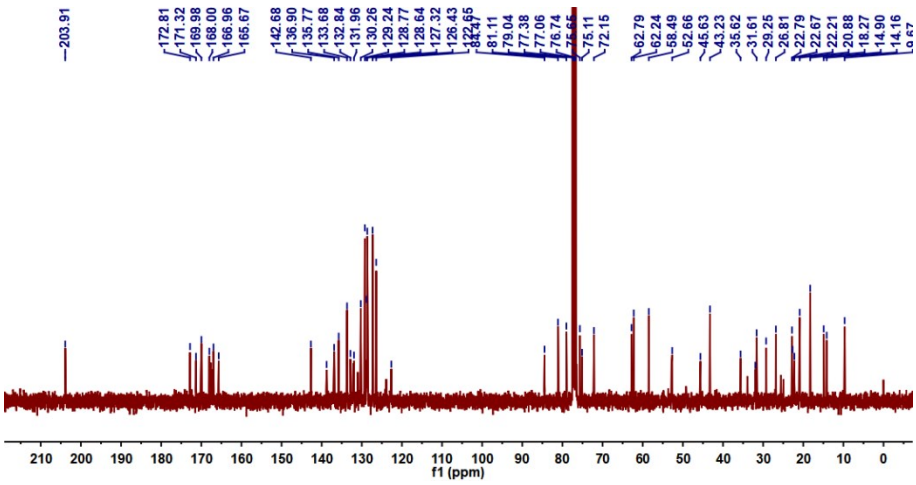

Fig. S15 The ^{13}C NMR spectrum of AMPTX in $CDCl_3$.

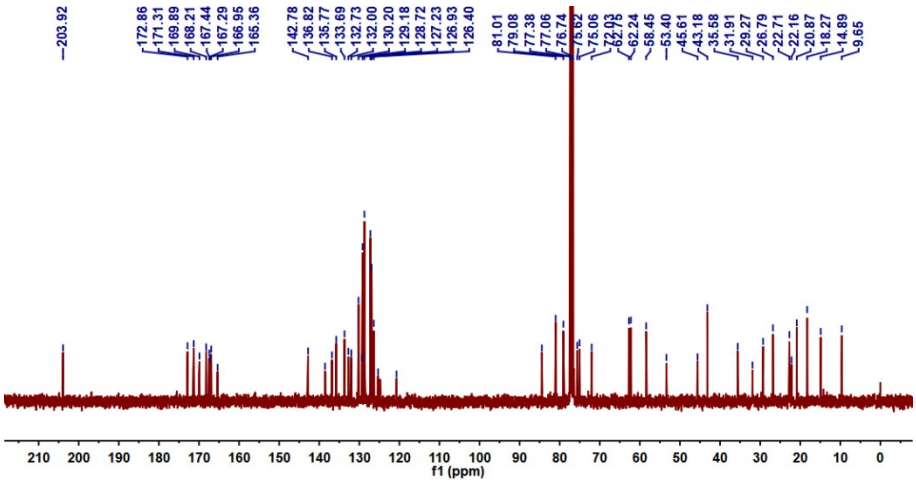

Fig. S16 The ^{13}C NMR spectrum of HPTX in $CDCl_3$.

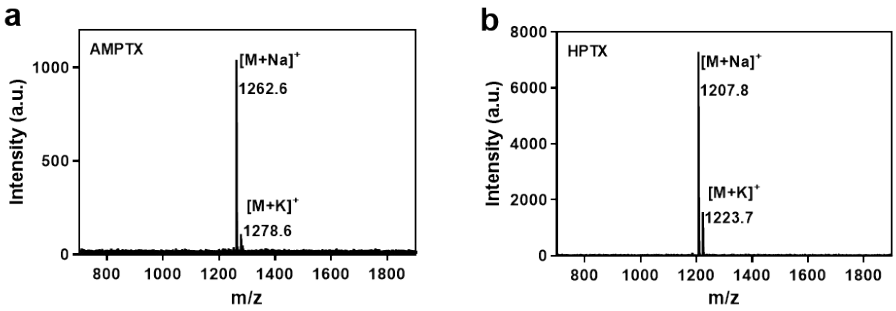

Fig. S17 The MALDI-TOF MS spectra of AMPTX (a) and HPTX (b).

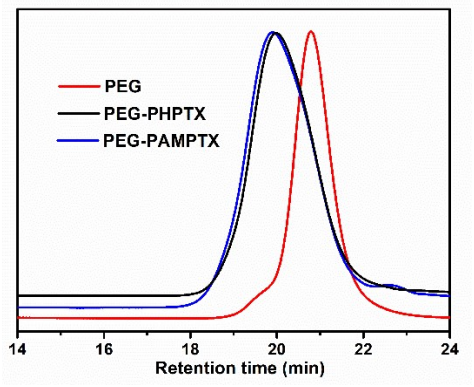

Fig. S18 The GPC traces of PEG-PAMPTX and PEG-PHPTX in DMF (50 °C, 0.8 mL/min).

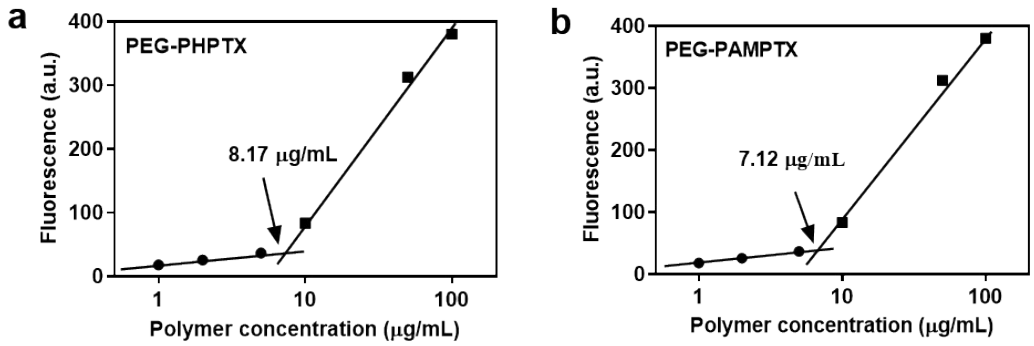

Fig. S19 CMC determination of PEG-PHPTX (a) and PEG-PAMPTX (b) micelles.

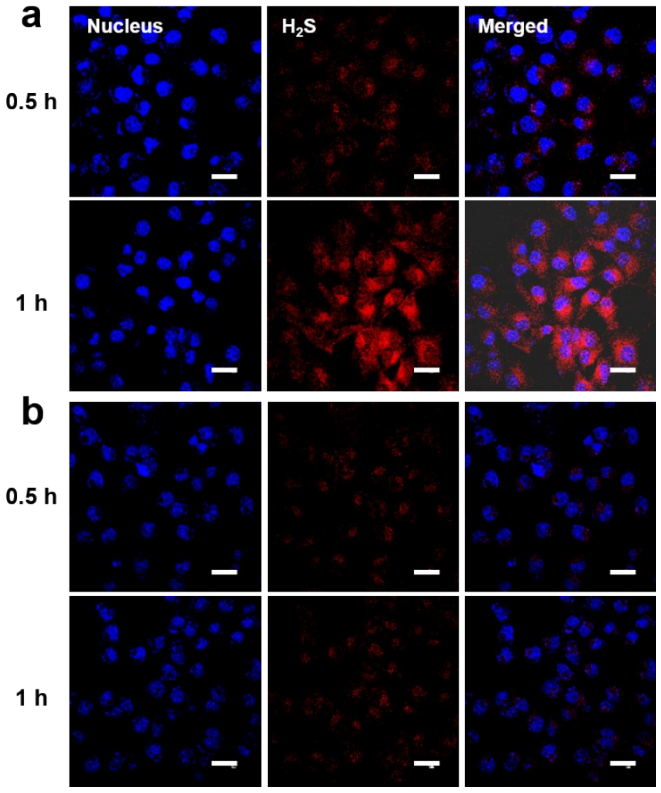

Fig. S20 Confocal images of intracellular H_2S generation in MDA-MB-231 breast cancer cells after incubation with (a) or without (b) SPRC. Cells were pretreated with 50 μM AzMC for 30 min before SPRC (1 mM) was added. Nuclei were stained by NucRed Live 647 Ready Probes Reagent shown in blue and H_2S were visualized by AzMC shown in red. Scale bar = 40 μm.

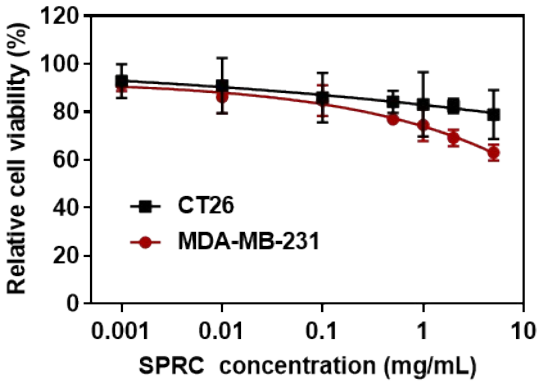

Fig. S21 The cytotoxicity of SPRC against CT26 and MDA-MB-231 cells.

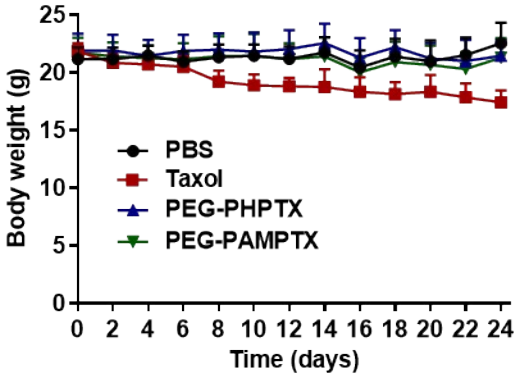

Fig. S22 The body weight variation of the tumor-bearing mice during the experiment.

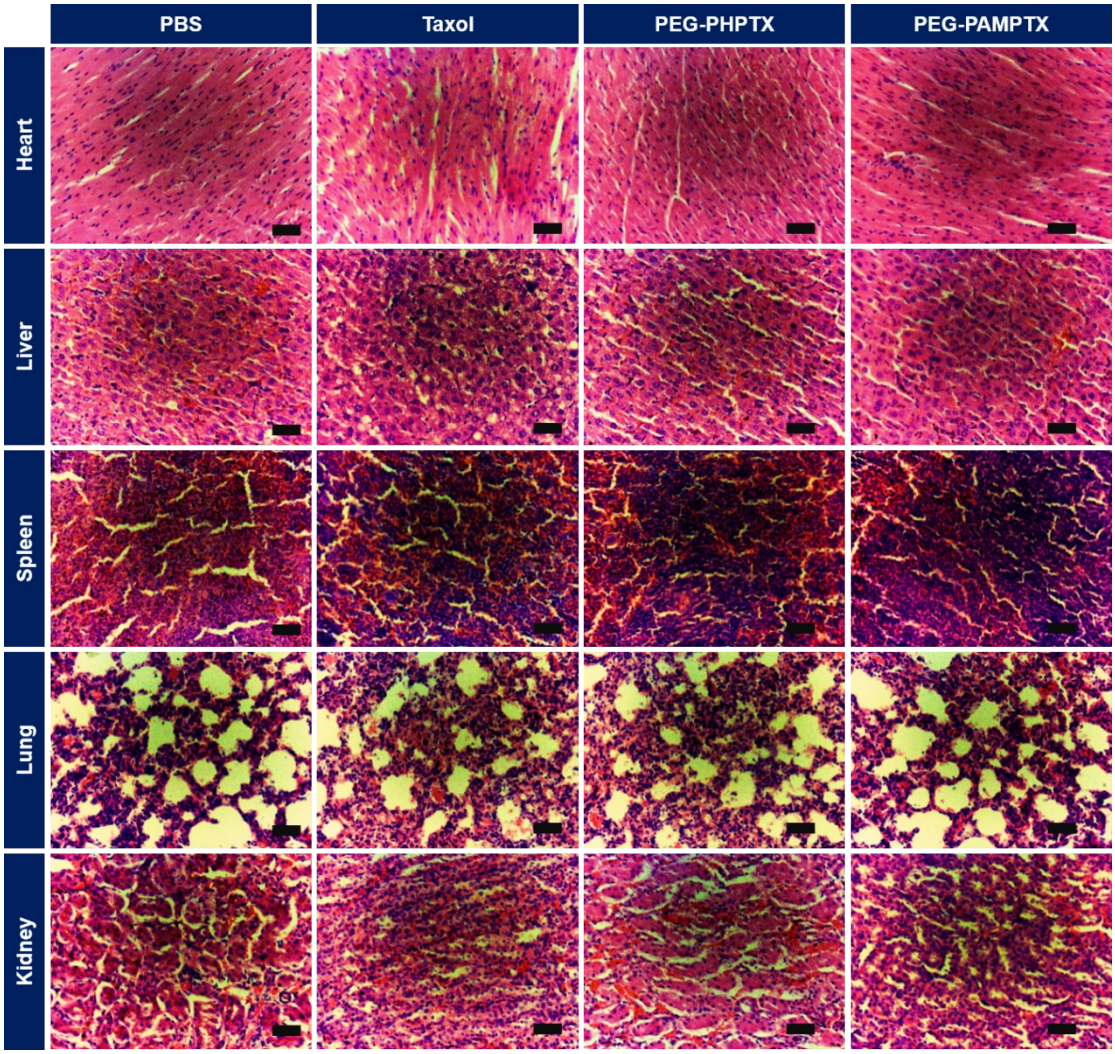

Fig. S23 Representative histological features of major organs from mice with MDA-MB-231 tumors. Tissue paraffin sections were 10-μm thick. The tissues sections were stained with hematoxylin-eosin and examined by light microscopy. Scale bars = 50 μm.

Table S1. The IC50 values of each formulation against different cell lines.

Formulations	IC50 (μg/mL)	
	MDA-MB-231	CT26
PEG-PAMPTX(SPRC-)	>100	4.69±0.12
PEG-PAMPTX(SPRC+)	0.10±0.05	0.63±0.07
PEG-PHPTX(SPRC-)	>100	46.19±2.56
PEG-PHPTX(SPRC+)	>100	73.14±3.67
PTX	5.20±0.6	0.14±0.03

References

1. M. Semsarilar, V. Ladmiral, A. Blanazs and S. P. Armes, Polym. Chem., 2014, 5, 3466-3475.